TOTAL COLIFORMS AND *E. COLI* MEMBRANE FILTRATION METHOD

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Sensitivity: 1 CFU/100 mL
m-ColiBlue24® Broth
U.S. Environmental Protection Agency (USEPA) Approved for Drinking Water
Method No. 10029
Revision 3, 2/13/02

1.0 Scope and Application

1.1 This method determines the presence or absence of total coliforms and *E. coli* in finished potable water using a selective and differential membrane filtration (MF) medium, m-ColiBlue24 Broth.

1.2 This method can detect the presence or absence of both total coliforms and *E. coli* simultaneously within 24 hours and without the need for a confirmation step.

1.3 The detection limit of the method is one colony forming unit (CFU) of coliform bacteria per 100 mL of sample. See Attachment 1.3.

2.0 Summary of Method

2.1 Coliform bacteria are identified in water either as total coliforms or *E. coli*. Total coliforms can be present in water without *E. coli* being present; *E. coli* cannot be present in water without total coliforms also being present.

2.2 A 100-mL volume of sample is filtered through a 47-mm membrane filter using standard techniques. The filter is then transferred to a 50-mm petri plate containing an absorbent pad saturated with m-ColiBlue24 Broth. The filter is then incubated at 35°C ± 0.5°C for 24 hours. If coliform bacteria are present in the sample, both red and blue colonies may appear; the blue colonies are specific to the presence of *E. coli*.

2.3 m-ColiBlue24 Broth is a nutritive, lactose-based medium, containing inhibitors to selectively eliminate growth of non-coliforms. It is analogous to an improved version of m-Endo. Total coliform colonies growing on the medium are highlighted by a non-selective dye, 2,3,5-Triphenoltetrazolium Chloride (TTC), which produces red colored colonies. Among the total coliform colonies, which grow up on the medium, any *E. coli* colonies are distinguishable by a selective blue color, resulting from the action of b-glucuronidase enzyme on 5-Bromo-4-Chloro-3-Indolyl-Beta-D-glucuronide (BCIG).

3.0 Definitions

3.1 Material Safety Data Sheet (MSDS) - Written information provided for each chemical reagent or standard about a chemical's toxicity, health hazards, physical properties, flammability, and reactivity. It also includes storage, spill, and handling precautions.

3.2 Total Coliform Bacteria - Bacteria belonging to the genera *Klebsiella* sp., *Enterobacter* sp., *Citrobacter* sp., or *Escherichia* sp.

3.3 Coliform Positive Colony - A red or blue colony.

3.4 Coliform Negative Colony - A clear or white colony.

3.5 *Escherichia coli* or *E. coli* Bacteria - A genus within the total coliform group typified by possession of the enzyme b-Glucuronidase, ability to grow at 44.5°C, and form indole from tryptophan.

3.6 *E. coli* Positive Colony - A blue colony.

3.7 *E. coli* Negative Colony - A non-blue colony.

4.0 Interferences

4.1 No interferences to the colony color development have been found in finished potable water samples. Similarly, particulates in water samples do not alter the efficacy of the medium, although excess particulates may cause colonies to grow together on crowded filters or slow the sample filtration process.

5.0 Safety

5.1 Standard safety practices appropriate to microbiology laboratories should be followed. Follow the test procedure carefully and observe all precautionary measures.

5.2 Solid and liquid waste materials containing or suspected to contain viable bacteria should be decontaminated using an autoclave or by using an appropriate disinfectant before discarding.

5.3 Refer to the appropriate Material Safety Data Sheets supplied for each reagent for comprehensive safety data essential to proper use.

6.0 Equipment and Supplies

6.1 Equipment

6.1.1 Air Incubator - Capable of operating at 35°C ± 0.5°C.

6.1.2 Vacuum pump.

6.1.3 Membrane filtration funnel unit and flask.

6.1.4 Dissecting microscope, capable of 10–15X magnification. The microscope should be equipped with a fluorescent illuminator.

6.2 Supplies/Glassware - Cleanse all glassware thoroughly with a suitable detergent and hot water, rinse with hot water to remove traces of detergent residual, and rinse again with laboratory-pure water. Sterilize all glassware by autoclaving 15 min. at 121°C or by heating in an oven for at least 1 hour at 170°C.

6.2.1 Presterilized 50-mm MF petri plates with pads.

6.2.2 45-mm presterilized membrane filters.

6.2.3 Sterile Forceps.

6.2.4 Sterile glass or plastic sample collection containers.

6.2.5 Sterile graduated cylinders.

6.2.6 Sterile pipets.

6.2.7 Sterile MF filtration unit.

6.2.8 Side-arm flask.

6.2.9 Biohazard bag.

6.2.10 150 mL of sterile rinse water.

7.0 Reagents and Standards

7.1 Growth Medium

7.1.1 The membrane filtration method for total coliforms and *E. coli* utilizes m-ColiBlue24 Broth, Hach product 26084-20, 26084-42, or 26084-50. The broth is provided in ready to use 2-mL ampules or 100-mL glass bottles. Under proper storage conditions (2–8°C) the broth has a shelf life of one year. The broth contains a nutritive medium and colorimetric indicators.

7.2 Dechlorinating Reagent

7.2.1 Hach decholorinating reagent Powder Pillow (14363-69) contains sodium thiosulfate ($Na_2S_2O_3$) and sodium sulfate (Na_2SO_4).

7.2.2 To make a 3% sodium thiosulfate ($Na_2S_2O_3$) solution, add 48.18 g of sodium thiosulfate pentahydrate to approximately 500 mL of deionized water, then dilute to 1 L with deionized water.

8.0 Sample Collection, Dechlorination, Preservation, Shipment and Storage

8.1 Water Sample Collection

8.1.1 Sample Collection Containers - Samples should be collected in sterile, clean glass or heat-resistant bottles. Presterilized Whirl-Pak®[1] Bags may also be used.

8.1.2 Sampling Procedure - Potable water samples are taken by first flushing the tap 2–3 minutes to clear service line. Collect samples using aseptic techniques to avoid contamination. For other water samples, aseptically collect water representative of the source.

8.2 Dechlorination - Water containing chlorine or other halogens must be treated with sodium thiosulfate to allow accurate evaluation of microbial content. Add one decholorinating reagent Powder Pillow (14363-69) by aseptically cutting off the tip of an alcohol-rinsed pillow and pouring the contents into 100 mL of chlorinated water sample. Alternatively, pipet 0.1 mL of a 3% sodium thiosulfate ($Na_2S_2O_3$) solution into 100 mL of a chlorinated water sample. Presterilized Whirl-Pak® Bags contain sufficient sodium thiosulfate powder to neutralize a 100-mL chlorinated water sample.

8.3 Preservation, Shipment, Storage - Samples should be tested as soon as possible. If analysis cannot be done within 1 hr of collection, potable water samples should be held on ice or at 2–8°C in a refrigerator for a maximum holding time of 30 hours (16.1). Non-potable samples should be held at <10°C for a maximum period of 8 hours from sampling to analysis.

9.0 Quality Control

9.1 m-ColiBlue24 Broth undergoes quality control (QC) testing at the time of manufacture. A Certificate of Analysis is included with every m-ColiBlue24 product, stating that the m-ColiBlue24 Broth, as received by the analyst, is ready for use in analyzing water samples by the membrane filtration procedure. Each laboratory should test quality control using the following procedure. Three ampules will be required, as well as membrane filtration equipment. The three ampules will be used as an uninoculated blank, a positive control for both total coliforms and *E. coli,* and a negative control.

9.2 The day before the QC procedure, inoculate three 10-mL Tryptic Soy Broth tubes with *Psuedomonas aeruginosa* (ATCC 27853). Incubate at 35°C ± 0.5°C for 24 ± 4 hours.

9.3 Aseptically open three ampules of m-ColiBlue24 Broth and saturate the pads in three MF petri dishes.

9.4 Filter 100 mL of sterile water and place filter on the first pad. This is the uninoculated blank.

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* Whirl-Pak® is a registered trademark and patented product of Nasco; U.S. Patent 2,973,131.

9.5 Prepare serial dilutions as described in Standard Methods 9222B (16.2). Prepare a serial dilution of the 24 hr. *P. aeruginosa* culture so that filtration of a 100-mL volume would theoretically yield 20–80 colonies. Filter the bacteria-containing diluent. After filtration, rinse the funnel with several 20–30-mL volumes of sterile rinse water. Place membrane filter into the second plate of m-ColiBlue24 Broth; this is the negative control. Prepare appropriate serial dilutions of the *E. coli* culture and filter. Rinse the funnel as previously indicated. Using this same membrane filter, serially dilute the *E. cloacae* culture, filter and rinse. This will introduce both *E. coli* cells and *E. cloacae* cells onto the same filter for a dual positive control. Incubate all three MF petri plates for 24 hours at 35°C ± 0.5°C. The uninoculated control should remain blank. The *P. aeruginosa* control should have no colonies. The positive control should have both blue (*E. coli*) and red (*E. cloacae*) colonies.

9.6 Colonies may be picked from membrane filters and inoculated into Lauryl Tryptose Broth (LTB), Brilliant Green Lactose Bile (BGLB), EC+MUG, or other media for further QC testing if desired.

10.0 Calibration and Standardization

10.1 No calibration or standardization of m-ColiBlue24 Broth is required.

10.2 All equipment used in Section 6 should be calibrated and maintained according to manufacturer's instructions.

11.0 Procedure

11.1 Test Procedure

11.1.1 Aseptically open an ampule containing m-ColiBlue24 Broth and pour the broth onto the pad in a 50-mm MF petri plate.

11.1.2 Place a sterile filter onto a sterile filter holder. Using sterile graduated cylinders and pipets, measure an appropriate sample aliquot. Pour water sample into reservoir funnel on filter holder and draw the water through the filter using a vacuum pump. Rinse the funnel with several 20–30-mL volumes of sterile rinse water. With sterile forceps, transfer the filter to a petri plate containing the pad saturated with m-ColiBlue24. Invert plate and incubate at 35°C ± 0.5°C for 24 hours.

11.2 Interpretation

11.2.1 Examine filters for colony growth. Colonies are typically readily visible, but a dissecting microscope may prove useful.

11.2.2 A red or blue colony is a Total Coliform Positive result. A clear or white colony is a Total Coliform Negative result. A blue colony is specifically an *E. coli* Positive result. A non-blue colony is an *E. coli* Negative result:

	Positive Result	Negative Result
Total Coliform	Red or Blue Colony	Clear or White Colony
E. coli	Blue Colony only	Non-Blue Colony

11.2.3 The incubation time is 24 hours. If no colonies are visible after 24 hours, the sample is free of total coliforms and *E. coli*.

12.0 Data Analysis, Calculations, Interpretation and Reporting

12.1 Presence/Absence

12.1.1 The presence of at least one red or blue colony indicates the sample is total coliform positive. The presence of at least one blue colony also indicates the sample contains *E. coli*. Absence of red or blue colonies indicates the sample contains no total coliforms or *E. coli*. No further analysis or calculation is required.

12.2 Quantitation

12.2.1 This method is approved by the USEPA for the purposes of determining the presence or absence of total coliforms and *E. coli* in drinking water. Since a protocol for evaluating the use of a new method for the enumeration of total coliforms and/or *E. coli* in drinking water does not exist, new methods, such as m-ColiBlue24, cannot be approved by the USEPA as quantitative methods for USEPA reporting purposes. However, we have included the enumeration protocol for other (non-USEPA reporting) purposes.

12.2.2 Coliform density determinations can often provide additional data for problem solving within the water plant or for analysis of source water samples. m-Coliblue24 Broth can be used to make density determinations.

12.2.3 If enumeration of total coliform and/or *E. coli* populations is desired, refer to Standard Methods 9222B for appropriate dilutions of the sample to filter so that 20–80 coliform colonies are present after incubation. For analysis of potable water samples, a standard sample of 100 mL is always required. Calculate the number of microorganisms per 100-mL sample also as described in Standard Methods 9222B.

13.0 Method Performance Characteristics

13.1 Specificity: The specificity of m-ColiBlue24 Broth for recovery of total coliforms and *E. coli* was evaluated. These experiments were conducted according to the EPA protocol of June 30, 1992 (16.3). For *E. coli*, the false positive error was 2.5% and the undetected target error was 0% (Section 17.0, Table 17.1). Overall agreement between m-ColiBlue24 and *E. coli* reference methods was 98.8%. For total coliforms, the false positive error was 26.8% and the undetected target error was 1.6% (Table 17.2). Using m-Endo as a comparison for total coliform recovery to m-ColiBlue24, m-Endo total coliform false positive error was 29.6% and the undetected target error was 3.4 % (Table 17.3). Overall agreement for total coliform recovery was 86.2% for m-ColiBlue24, and 85.7% with m-Endo. Although the total coliform false positive errors seemed large initially, review of the literature revealed that many existing media for total coliform recovery have similar false positive error rates (16.4 - 16.10).

13.2 Precision and Bias: Not applicable, as this protocol was conducted on a presence/absence basis. If individuals wish to use this medium for quantitative determinations they may use precision and bias calculations specified by ASTM (16.11).

14.0 Pollution Prevention

14.1 Base the quantity of chemicals purchased on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volume should reflect anticipated usage and reagent stability.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions, consult *Less is better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's department of Government Regulations and Science Policy, 1155 16th Street N.W., Washington D.C., 20036. Phone: (202) 872-4477.

15.0 Waste Management

15.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

15.2 See the MSDS for product composition information and further guidance on waste disposal.

15.3 For more information on laboratory waste management, consult *Waste Management Manual for Laboratory Personnel,* available from the American Chemical Society's department of Government Regulations and Science Policy, 1155 16th Street N.W., Washington D.C., 20036. Phone: (202) 872-4477.

16.0 References

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16.2 "American Public Health Association, American Water Works Association, Water Environment Federation. Microbiological Examination, Part 9000" in: Standard Methods for the Examination of Water and Wastewater, 18th ed., A.E. Greenberg, L.S. Clesceri, A.D. Eaton, eds. Washington, D.C., American Public Health Association, 1993.

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16.6 Covert T.C., L.C. Shadix, E.W. Rice, J.R. Haines, and R.W. Freyberg. 1989. Evaluation of the autoanalysis colilert test for detection and enumeration of total coliforms. Appl. Environ. Microbiol. 55:2443–2447.

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16.8 Jacobs, N.J., W. L. Zeigler, F.C. Reed, T.A. Stukel, and E.W. Rice.1986. Comparison of membrane filter, multiple-fermentation-tube, and presence-absence techniques for detecting total coliforms in small community water systems. Appl. Environ. Microbiol. 51:1007–1012.

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16.10 Sartory, D.P. and L. Howard. 11992. A medium detecting b-glucuronidase for the simultaneous membrane filtration enumeration of *Escherichia coli* and coliforms from drinking water. Lett. Appl.Microbiol. 15:273-276.

16.11 American Society for Testing and Materials. Determination of precision and bias applicable methods of committee D-19 on water. D 2777-86. Annual Book of ASTM Standards, Vol. 11.01. ASTM, Philadelphia, 1993.

17.0 Tables

17.1 *E. coli* recovery on m-ColiBlue24

Reference

	Positive	Negative	Total
Positive	234	6	240
Negative	0	250	250
Total	234	256	490

Sensitivity	100.0%
Specificity	97.7%
False Positive Error	2.5%
Undetected Target Error	0%
Overall Agreement	98.8%

17.2 Coliform recovery on m-ColiBlue24

Reference

	Positive	Negative	Total
Positive	183	67	250
Negative	2	248	250
Total	185	315	500

Sensitivity	96.8%
Specificity	80.1%
False Positive Error	29.0%
Undetected Target Error	3.2%
Overall Agreement	85.7%

Reference

	Positive	Negative	Total
Positive	149	61	210
Negative	5	245	250
Total	154	306	460

Sensitivity	96.8%
Specificity	80.1%
False Positive Error	29.0%
Undetected Target Error	3.2%
Overall Agreement	85.7%